

# Heterotic groups of onions (*Allium cepa* L.) for the development of low-pungency hybrids

## Grupos heteróticos en cebolla (*Allium cepa* L.) para el desarrollo de híbridos con baja pungencia



FÁTIMA ROSANGELA DE SOUZA SARAIVA<sup>1</sup>  
RITA CAROLINA DE MELO<sup>1,3</sup>  
PAULO HENRIQUE CERUTTI<sup>1</sup>  
JEFFERSON LUÍS MEIRELLES COIMBRA<sup>1</sup>  
DANIEL PEDROSA ALVES<sup>2</sup>  
ALTAMIR FREDERICO GUIDOLIN<sup>1</sup>

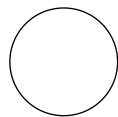
**Onion line: LA16.**

Photo: F.R. de S. Saraiva

### ABSTRACT

The growing demand for low-pungency cultivars onion has opened new market niches. The objective of this study was to characterize heterotic onion groups to develop low-pungency hybrid cultivars. A field experiment with a randomized block design and two factors (genotype and fertilization) arranged in split plots was carried out in Ituporanga-SC (Brazil), in 2019. The plots corresponded to two fertilization levels (with and without sulfur), and the subplots corresponded to 26 onion genotypes, where the germplasm was derived from different (male-sterile-LA and maintainer-LB) lines, advanced populations and cultivars. The bulbs were evaluated for the following traits: pyruvic acid (indicator of pungency), sulfur content and bulb yield. The genotype performance differed according to the fertilization level. In the sulfur-free treatments, four heterotic groups were identified. The genetic variation in the crosses LA11 × LB24 and LA15 × LB19 could be exploited for negative selection for pungency and positive selection for bulb yield.

**Additional key words:** bulb yield; ideotype; multivariate analysis; pyruvic acid content; standardized canonical coefficient.



<sup>1</sup> Universidade do Estado de Santa Catarina (UDESC), Lages (Brazil). ORCID Saraiva, F.R. de S.: 0000-0001-6466-7988; Melo, R.C. de: 0000-0002-5710-7621; Cerutti, P.H.: 0000-0001-6664-8449; Coimbra, J.L.M.: 0000-0001-9492-6055; Guidolin, A.F.: 0000-0003-3028-0958

<sup>2</sup> Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), Ituporanga (Brazil). Alves, D.P.: 0000-0003-4482-5082

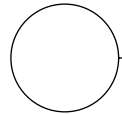
<sup>3</sup> Corresponding author. rita\_carol\_mel@hotmail.com

## RESUMEN

La creciente demanda por cultivares de cebolla de baja pungencia ha abierto nuevos nichos de mercado. El objetivo de este estudio fue caracterizar grupos heteróticos de cebolla para el desarrollo de cultivares híbridos con baja pungencia. Se realizó un experimento de campo con un diseño de bloques al azar con dos factores (genotipo y fertilización) dispuestos en parcelas divididas en Ituporanga-SC (Brasil), en 2019. Las parcelas correspondieron a dos niveles de fertilización (con y sin azufre) y las subparcela a vinteseis genotipos de cebolla, considerando germoplasma procedente de líneas androestéres-LA y mantenedoras-LB, poblaciones avanzadas y cultivares. Los bulbos fueron evaluados por las características: ácido pirúvico (indicador de pungencia), contenido de azufre y rendimiento de bulbo. Los genotipos revelaron un comportamiento diferencial según el nivel de fertilización adoptado. En la condición sin azufre cuatro grupos heteróticos fueron identificados, los cruzamientos LA11 × LB24 y LA15 × LB19 pueden generar variación genética y la respectiva selección negativa para la pungencia y positiva para la productividad de bulbos.

**Palabras clave adicionales:** rendimiento de bulbos; ideotipo; análisis multivariado; contenido de ácido pirúvico; coeficiente canónico estandarizado.

Received: 09-11-2021 Accepted: 01-03-2022 Published: 04-04-2022



## INTRODUCTION

The onion (*Allium cepa* L.) is one of the more important vegetable crops and is grown all over the world. Brazil produces approximately 1,165,402 t of onions on 54,179 properties, with an average yield of 29.6 t ha<sup>-1</sup>. Santa Catarina is the leading onion-producing state, with an output of 528.439 t and an average yield of 29.3 t ha<sup>-1</sup>, grown mostly by subsistence farmers (IBGE, 2020). Production is concentrated mainly in the region of Alto Vale do Itajaí, with 8,282 farms in Santa Catarina alone according to current estimates (EPAGRI/CEPA, 2019). Onion farmers are not only searching for higher-yielding cultivars but also for genotypes with differentiated chemical properties that could make onions a competitive product with high added (Porta *et al.*, 2014). In addition to health benefits, the onion has the ability to improve or accentuate the taste of other foods (Mallor *et al.*, 2011). So, low-pungency onions have been gaining popularity (Mallor and Sales, 2012; Coca *et al.*, 2013; Kim *et al.*, 2017; Yoo *et al.*, 2019, 2020). This onion type can be used for direct consumption without any processing. In the United States for example, they are consumed as “fresh fruit” or directly in salads.

In Brazil, low-pungency cultivars are not yet available. Thus, the development of low-pungency onions without losses in desirable characteristics such as bulb yield breeding programs should seek sources of and propose strategies to exploit genetic variability (Denre *et al.*, 2011; Cramer *et al.*, 2021; Lo Scalzo *et al.*, 2021). The onion is a sulfur-demanding crop (S).

This element is an important constituent of several amino acids, e.g., cystine, methionine, cysteine and tryptophan, as well as a precursor of volatile sulfur compounds. The content of S-based compounds determines the pungency level of onions (Tóth *et al.*, 2018). In onion fields, this element is frequently replaced by fertilization (in view of the high extraction of the nutrient) (Santos *et al.*, 2019). Thus, taking sulfur fertilization into account can reveal important interaction effects on variables of interest in genetic studies. The onion pungency level can be determined indirectly by measuring the pyruvic acid content (Wall and Corgan, 1992).

Because of the onion's reproductive system, breeding can be based on: *i*) population improvement where genetic and genotypic frequencies are determined (Falconer and Mackay, 1996) and selection for an increase in the frequency of favorable alleles is applied; and *ii*) hybrid breeding where the beneficial effects of heterosis are exploited. The hybrid-based approach is preferred with the efficiency of genetic-cytoplasmic male sterility systems in which crossing male-sterile with maintainer lines is a mandatory step. The main advantages of hybrid onion production are guaranteeing genetic consistency in the commercial product (bulbs) and homogeneity in seed germination.

The initial step in exploring the effects of heterosis is classifying populations into different heterotic groups. A heterotic group contains related or

unrelated genotypes, from the same germplasm or not, with a similar combining ability and a heterotic response when crossed with genotypes from other genetically distinct groups (Melchinger and Gumber, 1998). The more divergent the parents are, the higher the resulting genetic variability in the segregating population and the more likely the reassortment of alleles in new favorable combinations is because of the combination of contrasting characteristics in the parents. The progenies resulting from directed hybridizations have the complementarity of favorable alleles and open the way for selection in genetic improvement programs (Faria *et al.*, 2019; Khandagale and Gawande, 2019; Pevicharova *et al.*, 2020). The objective was to characterize heterotic groups among onion genotypes to produce hybrids that combine low pungency with an unaltered high bulb yield.

## MATERIAL AND METHODS

### Location, genetic constitutions and experiment units

This experiment was carried out from April to November of 2019 at the Company of Agricultural Research and Rural Extension of Santa Catarina (EPAGRI)/ Experimental Station Ituporanga, in Ituporanga-SC county (27°38' S, 49°60' W, at 475 m a.s.l.), Brazil. The soil was classified as medium-textured Cambissolo Húmico.

Twenty-six onion genotypes from the EPAGRI Onion Breeding (Tab. 1) were evaluated, which were derived from a germplasm of cultivars (8), lines (14) and improved populations (4). The A lines were male sterile and derived from backcrosses with maintainer lines. The B lines, on the other hand, were the result of selfing maintainer lines. Because of the high inbreeding depression after two or three selfings in onions, this paper used the term "line" for the resulting genotypes although they were not completely homozygous. The cultivars were representative of the onion cultivation conditions in Alto Vale do Itajaí, Santa Catarina, Brazil. The selection of the genotypes was based on their genetic variability for the traits of interest and on the adaptability of these genotypes to the growing region.

For each genotype, seedlings were produced according to the technological references proposed by the Onion Production System (EPAGRI, 2013). The fertilization of the seedling production beds consisted

**Table 1. Description of 26 onion genotypes (*Allium cepa* L.) based on their current breeding use. Identification according to the ABC hybridization system denotes the lines beginning with "A" (LA) as male-sterile and those beginning with "B" (LB) as maintainer lines.**

Genotype	Origin	Identification
Koreana	Cultivar	C1
Bela Vista	Cultivar	C2
Empasc 352 Bola Precoce	Cultivar	C3
SCS373 Poranga	Cultivar	C4
Epagri 363 SP	Cultivar	C5
P16000	Improved population	PA6
Valessul	Cultivar	C7
Bola precoce-Agroec.	Improved population	PA8
SCS379 Robusta	Cultivar	C9
SCS378 Pérola	Cultivar	C10
A16003-1638-23	Line	LA11
A16008-1616-20	Line	LA12
A16008-1616-22	Line	LA13
A16009-1606-21	Line	LA14
A16010-1601-22	Line	LA15
A16013-16232-23	Line	LA16
A16027-1650-24	Line	LA17
B16001-22	Line	LB18
B16006-21	Line	LB19
B16007-22	Line	LB20
B16030-21	Line	LB21
B16035-23	Line	LB22
B16133-23	Line	LB23
B16232-22	Line	LB24
P16001	Improved population	PA25
P16002	Improved population	PA26

of 0.5 kg m<sup>-2</sup> turkey manure (EP) and 200 g m<sup>-2</sup> NPK fertilizer 5-20-10 mixture. A phytosanitary treatment with conventional fungicides was applied following the manufacturer's recommendations. The sowing was carried out according to the earliness of the cultivar, where the later-maturing were sown earlier (April 8), and the earlier cultivars later (April 15). To understand the relationship of soil-applied sulfur and pungency, the sulfur application was controlled in the experiment. The experiment tested genotypes (26 levels) and sulfur fertilization (two levels, with and without). The complete block experiment with

two repetitions was arranged in a split plot design to optimize the fertilizer applications and improve control over the genotype effect. The fertilization was assigned to the plots, and the genotype was assigned to the subplots, resulting in a total of 104 plots, 52 per block.

The amount of mineral fertilizer was determined as recommended by the Soil Fertility and Chemistry Commission except for the sulfur fertilization (COF-SRS/SC, 2016). The fertilization consisted of 30 kg ha<sup>-1</sup> gypsum (CaSO<sub>4</sub>·2H<sub>2</sub>O, with 18.6% S), applied in the fertilization treatment before planting on July 12, 2019. The seedlings of each genotype were planted at 35 × 10 cm, resulting in a density of 285,000 plants/ha, according to the technical recommendations for the crop (Menezes Júnior, 2016). The plots contained seven rows, of which the five central ones (covering an area of 3 m<sup>2</sup>) were evaluated.

### Response variables

After a hailstorm on October 24, 2019, the plants were topped down, and the bulbs were harvested from all plots in November of 2019 and cured in the field. Thereafter, the pungency and sulfur were determined in a sample of four bulbs per treatment. The samples were prepared by removing the peel and inedible portions. The bulbs were cut longitudinally in four parts (1/4) to improve the representation of the bulb conditions. One quarter was minced in a juicer, and the resulting juice was frozen until analysis of the pyruvic acid. To determine the bulb sulfur content, another quarter was separated, placed in a paper bag, dried in an air circulation oven at 65°C to constant weight and ground in a knife mill.

The pungency content was obtained indirectly by measuring the pyruvic acid content in 2,4-dinitrophenylhydrazine reagent (DNPH) with the colorimeter method (Schwimmer and Weston, 1961). The pungency level was calculated based on the standard curve for sodium pyruvate (0 - 50 μmol g<sup>-1</sup>) and expressed in μmol g<sup>-1</sup>.

To determine the sulfur content in the bulbs, the samples were subjected to nitro-perchloric digestion, and S was determined in the extract with the turbidimetry method (in mg g<sup>-1</sup>) (Tedesco *et al.*, 1995). The other bulbs were left to cure in the field after harvest. Based on the transverse diameter (DT), the bulbs were classified, where those with a diameter greater

than 35 mm (classes 2, 3 and 4) were marketable. The bulb fresh weight was measured, and the bulb yield (BY) was determined (in t ha<sup>-1</sup>).

### Statistical analyses

The data were subjected to multivariate analysis of variance (MANOVA). This analysis considered the dependence between the response variables (covariance) (Hair *et al.*, 2009). The following mathematical model was applied:

$$Y_{ijkl} = m + B_i + A_j + G_k + A^*G_{jk} + e1_{ijkl} + e2_{ijkl} \quad (1)$$

Where,  $Y_{ijkl}$  was the values observed for the mean vectors in the  $l^{\text{th}}$  experiment unit in the  $i^{\text{th}}$  block in the  $j^{\text{th}}$  fertilization of the  $k^{\text{th}}$  genotype;  $m$  was the effect of the overall mean;  $B_i$  was the effect of the  $i^{\text{th}}$  level of the block factor;  $A_j$  was the effect of the  $j^{\text{th}}$  level of the fertilization factor;  $G_k$  was the effect of the  $k^{\text{th}}$  level of the genotype factor;  $A^*G_{jk}$  was the interaction effect of the  $j^{\text{th}}$  level of the fertilization factor with the  $k^{\text{th}}$  level of the genotype factor;  $e1_{ijkl}$  was the residual plot and  $e2_{ijkl}$  was the residual subplot effect.

After the global test of variance, a canonical discriminant analysis was performed to assist in the visualization of heterotic groups. Subsequently, multivariate contrasts were performed to test the formed groups. Standardized canonical coefficients for each comparison were obtained to identify the variables that contributed most to treatment discrimination. The canonical coefficients were interpreted as follows: positive values indicated the effect of treatment separation, while negative values were interpreted in the opposite direction (Hair *et al.*, 2009). All analyses were performed (SAS OnDemand for Academics, student version) with PROC GLM and PROC CANDISC.

## RESULTS AND DISCUSSION

The idea of improving a crop based on ideotypes is not new (Donald, 1968). The onion ideotype designed for this study should have the ability to produce above-reference levels of bulb yield, alongside lower levels of pyruvic acid (low pungency) in the same genetic background. A multivariate analysis has the advantage of taking the combined variation of numerous traits into consideration, in particular when these variables are related in a relevant dependency structure (Hair *et al.*, 2009). In this context, a significant effect of all treatment factors ( $P < 0.05$ ) was detected

with the multivariate analysis of variance (MANOVA) when considering pyruvic acid (PA), sulfur (S) and bulb yield (BY), simultaneously (Tab. 2).

For maximum exploitation of heterosis, parents must be chosen according to the germplasm origin from unrelated groups, commonly called heterotic groups (Melchinger and Gumber, 1998). Superior progenies in relation to the parents can be achieved with a cross between plants from different groups for the target trait. A study on genetic divergence proposed the analysis of morphological, agronomic and biochemical descriptors using multivariate methods, resulting in the indication of 13 groups from 64 evaluated onion accessions (Buzar *et al.*, 2007). Among these groups, varieties can be indicated for crosses and exploitation of heterosis.

Previous studies have emphasized the problem of sulfur applications for the pungency level (or pyruvic acid content) in onions. Some argue that the application of extra sulfur to soils with a high sulfur content does not increase pungency or related compounds in short-day onions (Lee *et al.*, 2009). Others have confirmed that the pungency level and sugar content of onions cannot be significantly altered by the soil type or by applying extra sulfur to soil with sufficiently high S levels (Hamilton *et al.*, 1998). However, it has been found that sulfur applications significantly increase pungency in chive (*Allium fistulosum* L.), with mean pyruvic acid contents of 2.34 and 6.76  $\mu\text{mol g}^{-1}$  for all cultivars grown at 0.01 and 4.00  $\text{mmol L}^{-1} \text{SO}_4^{2-}$ , respectively (Liu *et al.*, 2009). Brazilian genotypes must be genetically characterized with regard to the specific traits of the ideotype. The conditions of soil, management and climate are environment-specific, which would make a generalization of the crop performance unfeasible. This study addressed

the identification of heterotic groups based on bulb pungency and yield (Tab. 2).

The significant interaction effect indicated the differentiated performance of the genotypes in response to each fertilization level. In other words, a qualitative description of the interaction is required. Since the effect of greatest interest is on the genotypes, variations in environments, with and without fertilization, can be evaluated separately. This analysis may suggest the formation of different heterotic groups, depending on the environment in which the genotypes will be cultivated. The first canonical variable captured 80.03 and 73.33% of the accumulated variance without and with fertilization, respectively (Tab. 2). For a satisfactory interpretation of the variability in the genotypes, the first two canonical variables must represent 80% of the total variation in an analyzed data set (Cruz *et al.*, 2012). Thus, the contribution of agronomic traits can be identified, and the respective heterotic groups established satisfactorily with two dimensions (Fig. 1).

The canonical discriminant analysis visualized scores for each genotype according to the controlled fertilization levels. Also, by considering the germplasm source, the male-sterile (LA) and maintainer (LB) lines were separated (Fig. 1B and D) from the other genotypes to indicate crosses between heterotic groups that contain the male-sterile cytoplasm and the respective maintainers. According to the visual inspection, three and four groups were formed in the treatments without fertilization (Fig. 1A and B); while, the treatments with fertilization (Fig. 1C and D) only formed two groups.

For the characterization of the genotypes, this result indicated a buffering effect from the sulfur fertilization since a lower number of groups was established

**Table 2. Summary of multivariate analysis of variance using Wilks' Lambda test (U) in a split-plot experiment. The degrees of freedom of the numerator (DFN), denominator (DFL) and probability for the F test are indicated. Analysis considered the variables pyruvic acid (PA), sulfur (S) and bulb yield (BY).**

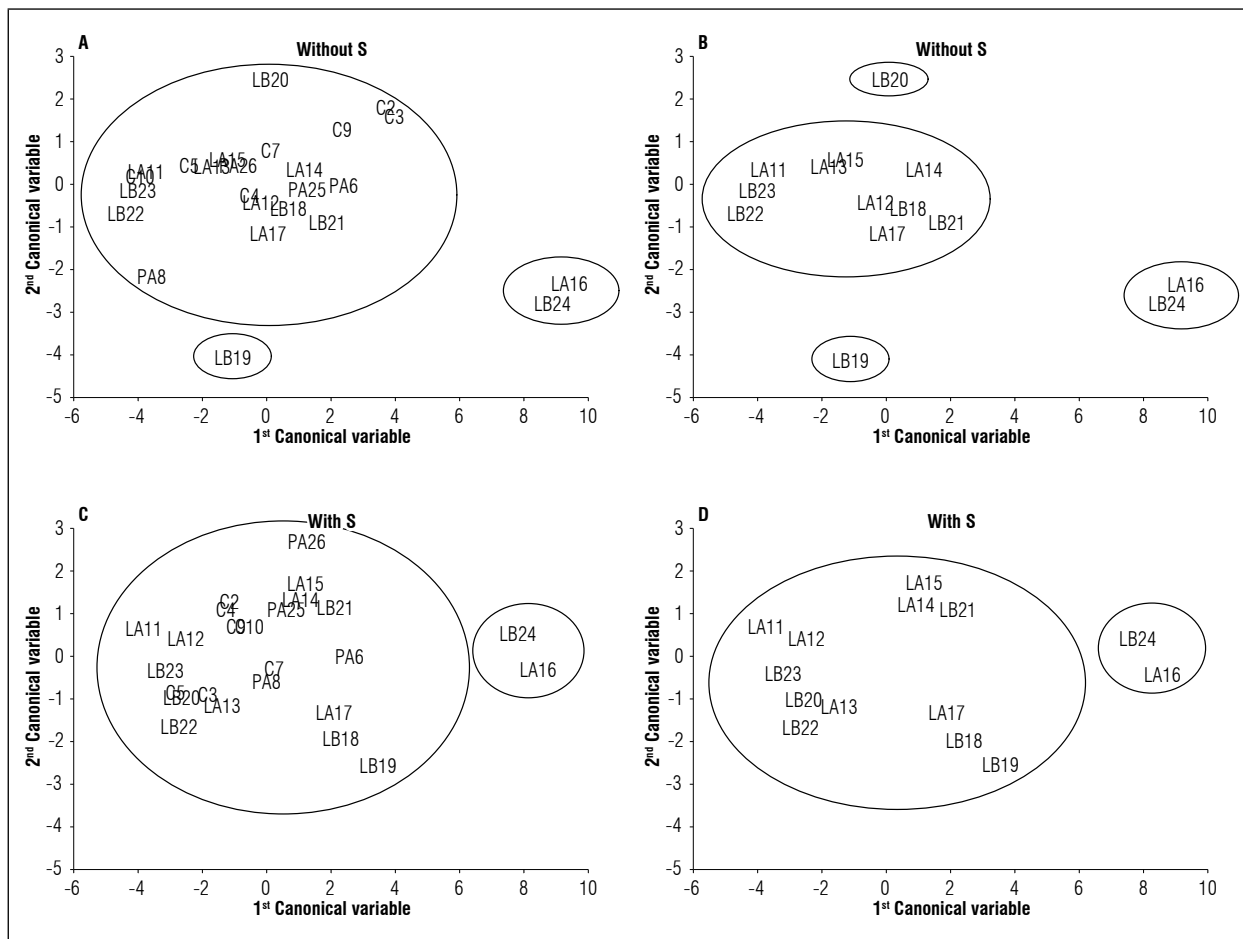
Source of variation	Error	DFN	DFL	U	$P > F$
Block	Plot	3	41	0.8909	0.1876
Fertilization (F)	Plot	3	41	0.6601	0.0006
Genotype (G)	Sub-plot	72	123.39	0.0092	0.0001
F * G	Sub-plot	72	123.39	0.1064	0.0008
i) Without F	VC (%)	$P > F$	ii) With F	VC (%)	$P > F$
	1 <sup>st</sup> - 80.03	0.0001		1 <sup>st</sup> - 73.33	0.0001
	2 <sup>nd</sup> - 93.73	0.0058		2 <sup>nd</sup> - 89.86	0.0014

in the environment where sulfur was supplied. This was also evident when the linear combinations of the canonical variables were analyzed. In the treatment without fertilization, the first canonical variable revealed that the function that most effectively differentiated the onion genotypes was  $Can1 = 0.469 \times PA + 51.994 \times S + 0.016 \times BY$ . In the fertilization treatment, the reference function consisted of  $Can1 = 0.043 \times PA + 36.745 \times S - 0.168 \times BY$ . This shows that the contribution of the variables was comparatively higher in the treatment without fertilization. When the element was supplied, the bulb yield was not significant for discriminating the accessions.

The potential genotypes for hybridization (male-sterile and maintainer lines) and clustering in the treatment without fertilization formed four heterotic groups (Fig. 1B). However, the discriminant

analysis formed groups based on a visual inspection, contrast-based mean comparison tests and standardized canonical coefficients, which were essential for comparing possible differences in performance between the genotypes and indicated which traits contributed more to the discrimination. (Tab. 3). Since the objective for establishing the heterotic groups was to form onion hybrids, comparisons were made between the male-sterile  $\times$  maintainer lines (LA  $\times$  LB). All male-sterile lines were compared with the maintainer lines LB19, LB20 and LB24 (contrasts C1 to C18), except for line LA16, which, because of its greater distance, was compared with all maintainer lines (contrasts C19 to C24), except LB24, which belonged to the same group.

Contrast C1 (LA11  $\times$  LB19) indicated significant differences between the mean vectors (Tab. 3). PA (0.04)



**Figure 1. Multivariate dispersion of 26 onion genotypes according to canonical scores for pyruvic acid (PA), sulfur (S) and bulb yield (BY): a) treatment without fertilization of any genotype; b) treatment without fertilization of male-sterile (LA) and maintainer (LB) lines only; c) treatment with fertilization of all genotypes; d) treatment with fertilization of male-sterile (LA) and maintainer (LB) lines only.**

and BY (1.54) contributed to the differences between the lines. Although the pungency levels of both lines were relatively lower than those of the other genotypes (7.42 and 7.03  $\mu\text{mol g}^{-1}$ ), BY was contrasting since line 11 produced three times the value of line 19. In other words, these genotypes belong to different heterotic groups, and if crossed, the beneficial effects of heterosis for pungency (negative selection) and bulb yield (positive selection) can be exploited.

Similarly, the comparison between lines LA11  $\times$  LB20 was significant (contrast C2), whereas pungency (-0.22) did not contribute to the observed difference (Tab. 3). In this case, the mean vector with an expressive contribution was S (2.28). Maintainer line LB20 had a sulfur content of 0.32  $\text{mg g}^{-1}$ , while the male-sterile line LA11 had 0.23  $\text{mg g}^{-1}$ . The genotype

performance differed in relation to sulfur uptake even in the treatment without fertilization, reinforcing the importance of understanding these relationships and establishing effective planning for plant selection.

Line LA11 also had a significant effect when compared to maintainer line LB24 (contrast C3). However, the traits that contributed most to discrimination were PA (0.54) and S (1.90). In this case, the pungency level of maintainer line LB24 was higher than that of the others (mean of 13.86  $\mu\text{mol g}^{-1}$ ), while that of male-sterile line LA11 was half of this value (mean of 7.42  $\mu\text{mol g}^{-1}$ ). In addition, the maintainer line had one of the lowest bulb yields of all evaluated genotypes.

The genetic bases that explain heterosis are based on the existence of heterozygous loci (Falconer and

**Table 3. Multivariate contrasts between onion genotypes (male-sterile lines vs. maintainer lines). Values of the Wilk Lambda test (U) and probability of F ( $P > F$ ) for each contrast and the respective standardized canonical coefficients for pyruvic acid (PA), sulfur (S) and bulb yield (BY).**

Comparison	U	P > F	PA	S	BY
C1: LA11 $\times$ LB19	0.59	0.0001	0.04	-1.09	1.54
C2: LA11 $\times$ LB20	0.66	0.0006	-0.22	2.28	0.22
C3: LA11 $\times$ LB24	0.30	0.0001	0.54	1.90	-0.84
C4: LA12 $\times$ LB19	0.77	0.0145	0.37	-0.89	1.73
C5: LA12 $\times$ LB20	0.79	0.0234	-0.70	1.13	1.19
C6: LA12 $\times$ LB24	0.44	0.0001	0.61	1.86	-0.84
C7: LA13 $\times$ LB19	0.68	0.0011	0.11	-0.50	1.71
C8: LA13 $\times$ LB20	0.82	0.0416	-0.45	2.00	0.63
C9: LA13 $\times$ LB24	0.36	0.0001	0.61	1.80	-0.90
C10: LA14 $\times$ LB19	0.65	0.0005	0.71	0.04	1.59
C11: LA14 $\times$ LB20	0.72	0.0034	1.21	-0.77	-0.51
C12: LA14 $\times$ LB24	0.45	0.0001	0.32	1.80	-1.06
C13: LA15 $\times$ LB19	0.70	0.0020	0.04	-0.22	1.76
C14: LA15 $\times$ LB20	0.88	0.1534	-0.38	1.88	0.90
C15: LA15 $\times$ LB24	0.38	0.0001	0.68	1.72	-0.92
C16: LA17 $\times$ LB19	0.81	0.0359	0.62	-0.07	1.63
C17: LA17 $\times$ LB20	0.72	0.0031	-0.76	0.89	1.25
C18: LA17 $\times$ LB24	0.48	0.0001	0.56	1.97	-0.76
C19: LA16 $\times$ LB18	0.49	0.0001	0.68	1.89	-0.73
C20: LA16 $\times$ LB19	0.54	0.0001	0.86	2.06	0.06
C21: LA16 $\times$ LB20	0.38	0.0001	0.79	1.38	-1.06
C22: LA16 $\times$ LB21	0.56	0.0001	0.72	1.87	-0.71
C23: LA16 $\times$ LB22	0.28	0.0001	0.53	2.07	-0.65
C24: LA16 $\times$ LB23	0.28	0.0001	0.56	2.00	-0.71

Mackay, 1996). The more distant and homozygous the populations involved in the cross are, the greater the expectations for the exploitation of hybrid vigor is (resulting in greater heterozygosity in the  $F_1$  population). Since the onion is affected by inbreeding depression, varieties maintain homozygous recessive alleles that are hidden by heterozygosity. Thus, in some crosses, hybrids with lower values of pyruvic acid can be detected.

The male-sterile lines LA12 and LA13 had the same behavior pattern as LA11 when crossed with the maintainer lines (contrasts C4 to C9). The contribution of bulb yield was notable in the discrimination of treatments when lines LB19 and LB20 were involved and in the pungency trait when lines LB19 and LB24 were involved. The male-sterile lines LA11, LA12 and LA13 probably not only belonged to the same heterotic group but were also improved in the same population, constituting only different selections of the same open-pollination variety, for example.

In all comparisons involving line LA14, trait PA contributed significantly to treatment discrimination (contrasts C10 to C12) (Tab. 3). Line LA14 had a  $5.0 \mu\text{mol g}^{-1}$  higher pyruvic acid content than lines LB19 and LB20. For contrast C10, all traits contributed to treatment discrimination, mainly bulb yield, with the highest effective contribution (1.59). On the other hand, the only comparison with no significant difference was contrast C14 ( $P=0.1534$ ). Although located in different heterotic groups, the multivariate scores of lines LA15 and LB20 were closely related, which may explain the non-significance of this comparison. This reinforces the importance of making comparisons based on probability values, rather than only on decisions based on visual inspection. In the comparisons C13 to C15, line LA15 had the lowest pungency content of all male-sterile lines, both in the treatment without ( $6.83 \mu\text{mol g}^{-1}$ ) and in the treatment with fertilization ( $5.80 \mu\text{mol g}^{-1}$ ). In other words, this line was not responsive to this environmental factor, which makes it very desirable for breeding programs for the selection of low-pungency genotypes.

The contrasts involving the male-sterile line LA16 were all significant (C19 to C24). Despite the differentiation of the lines by pungency and sulfur in the bulbs, they were close in relation to the trait bulb yield (Tab. 3). In addition, in contrast to LA15, line LA16 had the highest pyruvic acid content ( $14.08 \mu\text{mol g}^{-1}$ ) of all studied genotypes.

The National Onion Labs Inc. (NOL) in the United States certifies onion genotypes according to the pyruvic acid content (following the same protocol as in this study), with levels between 0 and  $5.5 \mu\text{mol g}^{-1}$  for low to medium-pungency onions. The germplasm of this study was comparatively more pungent than the above levels. A study carried out in Chapadão do Lageado (Alto Vale do Itajaí-SC), where the same analysis method was used, reported a variation in pungency from 4.84 to  $7.61 \mu\text{mol g}^{-1}$  (Schunemann *et al.*, 2006). A cultivar common to the germplasm of both studies was Bola Precoce, for which a lower pyruvic acid content ( $5.80 \mu\text{mol g}^{-1}$ ) was detected in our study ( $10.16 \mu\text{mol g}^{-1}$ ). Possibly, the local conditions and sulfur previously available in the soil prior to fertilization may have significantly increased the pungency of the onion genotypes tested in Ituporanga-SC.

Generating new genotypes to meet current consumer demands is one of the major challenges for breeding, more so, when no information is available for the trait of interest within the accessions available in the program. Consequently, the characterization must be initiated. However, considering more than one trait simultaneously and thus the existing covariation between the response variables that make up the ideotype can significantly improve efficiency and reduce the time invested for genotype selection. In addition, it would make no sense to add a different characteristic to a crop (low pungency) without maintaining the expected levels of bulb yield (in this case). This study established, within the available genetic variation in the EPAGRI onion germplasm, that four heterotic groups can be recombined in the future to select the best genetic background. Some of the suggested hybridizations are *i*) crosses of low-pungency and contrasting genotypes for the trait bulb yield (LA11  $\times$  LB19) (Tab. 3). *ii*) crosses of genotypes contrasting for the trait pungency, but similar in bulb yield (LA11  $\times$  LB24), *iii*) crosses of male-sterile lines with greater pungency than the maintainer line (LA14  $\times$  LB19) and *iv*) crosses of not very pungent male-sterile lines that are stable regardless of the cultivation system (LA15  $\times$  LB19).

## CONCLUSIONS

This study established, within the available genetic variation in the EPAGRI onion germplasm, that four heterotic groups can be recombined in the future to select the best genetic background.



**Conflict of interests:** This manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the results.

## BIBLIOGRAPHIC REFERENCES

- Buzar, A.G.R., V.R. Oliveira, and L.S. Boiteux. 2007. Estimativa da diversidade genética de germoplasma de cebola via descritores morfológicos, agronômicos e bioquímicos. *Hortic. Bras.* 25(4), 527-532. Doi: 10.1590/s0102-05362007000400007
- Coca, A.C., C.E. Carranza, D. Miranda, and M.H. Rodríguez. 2013. Efecto del NaCl sobre los parámetros de crecimiento, rendimiento y calidad de la cebolla de bulbo (*Allium cepa* L.) bajo condiciones controladas. *Rev. Colomb. Cienc. Hortic.* 6(2), 196-212. Doi: 10.17584/rcch.2012v6i2.1977
- COFSRS/SC, Comissão de Química e Fertilidade do Solo – RS/SC Brazil. 2016. Manual de adubação e de calagem para os estados do Rio Grande do Sul e de Santa Catarina. 11<sup>th</sup> ed. Sociedade Brasileira de Ciência do Solo, Porto Alegre, Brazil.
- Cramer, C.S., S. Mandal, S. Sharma, S.S. Nourbakhsh, I. Goldman, and I. Guzman. 2021. Recent advances in onion genetic improvement. *Agronomy* 11(3), 482. Doi: 10.3390/agronomy11030482
- Cruz, C.D., A.J. Regazzi, and P.C.S. Carneiro. 2012. Modelos biométricos aplicados ao melhoramento genético. 4<sup>th</sup> ed. Universidade Federal de Viçosa, Viçosa, Brazil.
- Denre, M., S. Pal, A. Chattopadhyay, D. Mazumdar, A. Chakravarty, and A. Bhattacharya. 2011. Antioxidants and pungency of onion. *Int. J. Veg. Sci.* 17(3), 233-245. Doi: 10.1080/19315260.2010.543452
- Donald, C.M. 1968. The breeding of crop ideotypes. *Euphytica* 17(3), 385-403. Doi: 10.1007/BF00056241
- EPAGRI, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina Brazil. 2013. Sistema de produção para a cebola - Santa Catarina. 4<sup>th</sup> ed. Florianópolis, Brazil.
- EPAGRI/CEPA, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina/Centro de Socioeconomia e Planejamento Agrícola Brazil. 2019. Síntese Anual da Agricultura de Santa Catarina 2018-2019. Vol. 1. Florianópolis, Brazil.
- Falconer, D.S. and T.F.C. Mackay. 1996. Introduction to quantitative genetics. 4<sup>th</sup> ed. Prentice Hall, Harlow, UK
- Faria, M.V., W.L. Zaluski, J. Rosa, E.S. Rossi, J. Resende, R.F. Kobori, R.L. Santos, and P.R. Da-Silva. 2019. Genetic divergence among inbred onion lines and correlation with heterosis and combining ability. *Genet. Mol. Res.* 18(3), gmr18316. Doi: 10.4238/gmr18316
- Hair, J.F.J., W.C. Black, B.J. Babin, R.E. Anderson, and R.L. Tatham. 2009. Análise multivariada de dados. 6<sup>th</sup> ed. Bookman, São Paulo, Brazil.
- Hamilton, B.K., K. Sun Yoo, and L.M. Pike. 1998. Changes in pungency of onions by soil type, sulphur nutrition and bulb maturity. *Sci. Hortic.* 74(4), 249-256. Doi: 10.1016/S0304-4238(98)00089-2
- IBGE, Instituto Brasileiro de Geografia e Estatística Brazil. 2020. Índice nacional de preços ao consumidor-INPC/Séries históricas. In: <https://www.ibge.gov.br/estatisticas/economicas/precos-e-custos/9258-indice-nacional-de-precos-ao-consumidor.html?edicao=26615&t=series-historicas>; consulted: September, 2021.
- Kim, H.Y., D. Jackson, K. Adhikari, C. Riner, and G. Sanchez-Brambila. 2017. Relationship between consumer acceptability and pungency-related flavor compounds of *Vidalia* onions. *J. Food Sci.* 82(10), 2396-2402. Doi: 10.1111/1750-3841.13915
- Khandagale, K. and S. Gawande. 2019. Genetics of bulb colour variation and flavonoids in onion. *J. Hortic. Sci. Biotechnol.* 94(4), 522-532. Doi: 10.1080/14620316.2018.1543558
- Lo Scalzo, R., M. Fibiani, V. Picchi, and B. Parisi. 2021. Low pungency and phytochemicals relationship during bulb assessment in the sweet onion breeding program. *Sci. Hortic.* 285(2021), 110191. Doi: 10.1016/j.scienta.2021.110191
- Lee, E.J., K.S. Yoo, J. Jifon, and B.S. Patil. 2009. Application of extra sulfur to high-sulfur /soils does not increase pungency and related compounds in shortday onions. *Sci. Hortic.* 123(2), 178-183. Doi: 10.1016/j.scienta.2009.09.009
- Liu, S., H. He, G. Feng, and Q. Chen. 2009. Effect of nitrogen and sulfur interaction on growth and pungency of different pseudostem types of Chinese spring onion (*Allium fistulosum* L.). *Sci. Hortic.* 121(1), 12-18. Doi: 10.1016/j.scienta.2009.01.019
- Mallor, C., M. Balcells, F. Mallor, and E. Sales. 2011. Genetic variation for bulb size, soluble solids content and pungency in the Spanish sweet onion variety Fuentes de Ebro. Response to selection for low pungency. *Plant Breed.* 130(1), 55-59. Doi: 10.1111/j.1439-0523.2009.01737.x
- Mallor, C. and E. Sales. 2012. Yield and traits of bulb quality in the Spanish sweet onion cultivar 'Fuentes de Ebro' after selection for low pungency. *Sci. Hortic.* 140, 60-65. Doi: 10.1016/j.scienta.2012.04.003
- Melchinger, A.E. and R.K. Gumber. 1998. Overview of heterosis and heterotic groups in agronomic crops. pp. 29-44. In: Larnkey, K.R. and J.E. Staub (eds.). Concepts and breeding of heterosis in crop plants. Vol. 25. Crop Science Society of America, Madison, WI. Doi: 10.2135/cssaspecpub25.c3

- Menezes Júnior, F.O.G. 2016. Aspectos fitotécnicos. pp. 41-48. In: Menezes Júnior, F. and L.L. Marcuzzo (Orgs.). Manual de boas práticas agrícolas: guia para sustentabilidade das lavouras de cebola do Estado de Santa Catarina. EPAGRI, Florianópolis, Brazil.
- Pevicharova, G., S. Genova, E. Nacheva, and N. Penov. 2020. Heterosis expression toward technological parameters in F1 onion hybrids for drying. *J. Cent. Eur. Agric.* 21(2), 338-343. Doi: 10.5513/JCEA01/21.2.2487
- Porta, B., M. Rivas, L. Gutiérrez, and G.A. Galván. 2014. Variability, heritability, and correlations of agronomic traits in an onion landrace and derived S1 lines. *Crop Breed. Appl. Biotechnol.* 14(1), 29-35. Doi: 10.1590/s1984-70332014000100005
- Santos, C.A.E., J.E. Yuri, and N.D. Costa. 2019. Efeito do enxofre na produtividade e no teor de ácido pirúvico em cultivares de cebola. *Embrapa Semiárido*, Petrolina, Brazil.
- Schunemann, A.P., R. Treptow, D.L. Leite, and J.L. Vendruscolo. 2006. Pungência e características químicas em bulbos de genótipos de cebola (*Allium cepa* L.) cultivados no Alto Vale do Itajaí, SC, Brasil. *Rev. Bras. Agrociênc.* 12(1), 77-80.
- Schwimmer, S. and W.J. Weston. 1961. Onion flavor and odor, enzymatic development of pyruvic acid in onion as a measure of pungency. *J. Agric. Food Chem.* 9(4), 301-304. Doi: 10.1021/jf60116a018
- Tedesco, M.J., C. Gianello, C.A. Bissani, H. Bohnen, and S. Volkweiss. 1995. Análises de solo, plantas e outros materiais. *Bol. Tec.* 5. 2<sup>nd</sup> ed. UFRGS, Porto Alegre, Brazil.
- Tóth, T., J. Bystrická, J. Tomáš, P. Siekel, J. Kovarovič, and M. Lenková. 2018. Effect of sulphur fertilization on contents of phenolic and sulphuric compounds in onion (*Allium cepa* L.). *J. Food Nutr. Res.* 57(2), 170-178.
- Wall, M.M. and J.N. Corgan. 1992. Relationship between pyruvate analysis and flavor perception for onion pungency determination. *HortScience* 27(9), 1029-1030. Doi: 10.21273/hortsci.27.9.1029
- Yoo, K.S., D. Leskovar, B.S. Patil, and E.J. Lee. 2019. Effects of leaf cutting on bulb weight and pungency of short-day onions after lifting the plants. *Sci. Hortic.* 257, 108720. Doi: 10.1016/j.scienta.2019.108720
- Yoo, K.S., L.M. Pike, B.S. Patil, and E.J. Lee. 2020. Developing sweet onions by recurrent selection in a short-day onion breeding program. *Sci. Hortic.* 266, 109269. Doi: 10.1016/j.scienta.2020.109269